

# Measuring cortical thickness with MRI in a transgenic animal model of AD

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**Abstract:** Alzheimer’s Disease (AD) is a progressive age-related neurodegenerative disorder, which can be studied through the use of transgenic animal models. AD is characterized by a loss of brain gray matter, resulting in changes in the cortical thickness that can be measured from Magnetic Resonance Imaging (MRI). The present work aimed to measure the cortical thickness from rat brain MRI by creating an automated tailored program implemented in Python and using available algorithms and in-house designed functions. The program was further used to analyse the temporal evolution of the cortical thickness in a transgenic AD model compared to control wild-type rats.

## I. INTRODUCTION

Advanced Image Processing is a key tool to obtain measures from Magnetic Resonance Imaging (MRI) and to study neurological diseases.

In this sense, animal models of Alzheimer’s disease (AD) are essential to understanding the disease progression. AD is a progressive age-related neurodegenerative disease that has become the most common form of dementia in elderly populations [1].

In our case we aimed to measure the brain cortical thickness as it appears to be a suitable marker to study the disease progression [2]. Thus, studying the cortical thickness we expected to observe differences between TgF344-AD rats, which are a transgenic model of AD [3], and another group of wild-type littermates used as control (CTR). Using MRI, the cortical thickness can be measured as the distance between the white matter and the pial boundaries [2].

The rat cortex is a lissencephalic layer, formed by neuron bodies and, given the similarities within inbred strains, it is almost identical among individuals, which simplifies the creation of a cortical thickness algorithm considerably. The ability to compute accurate registrations implies that the definition of the cortex and its boundaries can be created by mapping a pre-existing atlas to each animal [4]. For this reason, our choice was a volume-based cortical thickness algorithm with boundary definitions.

The objective of this study was to design a framework for automatically measuring cortical thickness from rat brain MRI and to use it to obtain individual measures as well as group differences between AD and CTR groups of rats. The algorithm was designed in Python and could be easily adapted to study other rat populations.

## II. DATA

The data acquisition was not part of this project. It was previously performed following the Catalan and European legislation and with the approval of the Exper-

imental Animal Ethical Committee of the University of Barcelona.

The experiment was carried out using a cohort of 19 rats, including 9 transgenic TgF344-AD rats and 10 CTR rats. These animals underwent MRI at five different times: t1( $170 \pm 43$  days), t2( $246 \pm 21$  days), t3( $338 \pm 9$  days), t4( $447 \pm 17$  days) and t5( $534 \pm 16$  days). In our study, some time points were missing for specific subjects due to different acquisition issues.

The MRI acquisition was conducted on a 7.0-T BioSpec 70/30 horizontal animal scanner. It included T2-weighted images (T2w), which were acquired with a RARE (rapid acquisition with relaxation enhancement) sequence, with effective echo time  $TE = 35.3$  ms,  $TR = 6000$  ms and RARE factor 8. The T2w images were processed to obtain a brain parcellation based on an atlas, as explained elsewhere [1].

## III. IMAGE PROCESSING METHOD

The input images for this project were a set of preprocessed data including the images of the brain (T2w) and the images of the region parcellation (labels) obtained from the T2w images. The rat brain atlas used to define the labels was a combination of atlases published previously, region names are based on previous research [5].

The proposed method estimates the thickness of the entire cortical manifold. The basic procedure was as follows: (A) Segmentation of the cortex from the labels images. (B) Mapping of the cortical boundaries to each of the individual rats. (C) Estimation of the cortical thickness. (D) Statistics to determine the differences in cortical thickness between groups of rats.

### A. Cortex Segmentation

For the study we were only interested in the cortical regions, so we only needed the mask with the cortex areas from the labels image. We used the FSL program (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki>) to obtain it

by keeping only the labels corresponding to cortical regions. We obtained a mask of the cortex, which indicated the approximate extend of the cortex but did not adapt correctly to the cortical borders. We created two versions of the cortical mask by applying an erode and a dilate operation to it. Therefore, we used these two masks as superior and inferior limits within our algorithm to find the actual contour.

The algorithm was applied slice by slice and only in the slices where the cortex was well defined and measurable. Therefore, the selection of the slices of each image was done by a threshold. Thus we divided the voxels of the cortex label by all the brain voxels of the current slice, this value was what we used for the threshold. We worked with left and right regions separately.

### B. Boundaries of the cortex

The technique used for estimating cortical thickness involved the localization of the inside and outside boundaries of the cortical surface, and then the computation of the distance between them as an estimations of the cortical thickness. Firstly, we mapped the exterior contour since we used it as a reference to get the interior contour. In both cases, the input image to the algorithm was the T2w image of the brain (Fig.3a). However, the algorithm was different for the interior and exterior boundaries. The following procedure was developed in Python using the scikit-image library:

For the exterior boundaries :

1. We obtained the mask of the brain by binarizing the T2w image of the brain (Fig.3a).
2. Before getting the exterior contour, we needed to fill the possible holes, which could remain inside the brain mask. We filled them with the function *reconstruction* using the option *erosion*.
3. We mapped the contour with the option *find contour*. This option uses the marching squares method [6]. In our case, the output were the coordinates of the exterior contour of the brain (Fig.1a).
4. We applied the dilated mask to the coordinates obtained in order to keep only the exterior contour coordinates of the selected side (Fig.1b).
5. We found the local maximum peaks of the image as coordinates, with the option *peak local max*. The peaks were the local maximum in a region of  $2(d_{min}) + 1$ , so the peaks were separated by at least the minimum distance. Finally, we had the outside contour of the cortex (Fig.1c).

For the interior boundaries:

1. We applied to the brain image (Fig.3a) an edge detector algorithm using Canny algorithm [7]. The selected width for the Gaussian was  $\sigma = 2$ , as

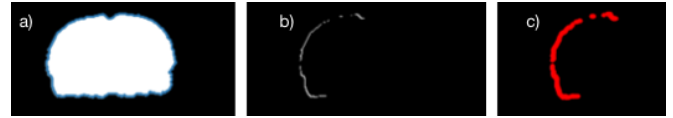


FIG. 1: Process applied to get the outside cortex contour. a) The brain mask mapped with the coordinates of the exterior contour. b) The outside cortex contour. c) The outside cortex contour mapped with the coordinates of the local maximum peaks.

we did not have so much noise we applied this medium value possible as the most suitable option. Thus, we obtained a binary edge map of the brain (Fig.2a).

2. We applied a dilatation to the exterior contour and we subtract this from the binary edge map of the brain (Fig.2a). The dilatation was applied to ensure that we subtracted all the points which corresponded to the exterior contour. Also, we had to apply a median filter as the image could have some spurious points remaining in the inside of the cortex. These points could affect the measurement of the distance between the inside and outside cortex. Finally, we obtained the image shown in Fig.2b.
3. We multiplied the image (Fig.2b) by the image of the dilated mask of the brain. In order to select the interior contour of the cortex.
4. We found the local maximum peaks, following the same method as with the exterior contour. And finally, we obtained the coordinates of the inside contour of the cortex (Fig.2c).

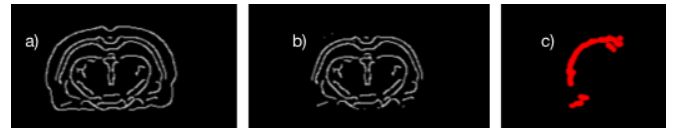


FIG. 2: Process applied to get the inside cortex contour. a) The edge map of the brain. b) The edge map of the brain without the outside contour. c) The inside cortex contour mapped with the coordinates of the local maximum peaks.

Following these steps we obtained the boundaries of the cortex (Fig.3b), which was the last step before measuring the cortical thickness.

### C. Estimation of the Cortical Thickness

Using the coordinates from the outside and inside cortex boundaries we measured the euclidean distance between them. We estimated the distance from each point of the outside contour as the minimum distance considering all the points of the inside contour (Fig.3c). How-



FIG. 3: Process applied to get the cortical thickness. a) The image of the brain in T2w. b) The image of the brain with the boundaries mapped. c) The image of the brain with one of the pairs of points which we use to measure the cortical thickness mapped.

ever, not all this values were correct, for example we obtained non-perpendicular lines. Since some of the exterior points targeted to the same interior point (Fig.4), as there were more points in the exterior contour than in the interior contour, this fact could introduce errors to the result. For this reason, we further checked if different exterior points were targeting the same interior point. We considered the correct cortical thickness by taking the point with the minimum euclidean distance, which would correspond to the more perpendicular line between both contours.



FIG. 4: Schematic representation of point correspondence. Blue line is the perpendicular line, which is the correct value of the cortical thickness. Yellow lines are the non-perpendicular lines, which are the incorrect values that we have to remove.

These values of the distance were in voxels so we needed to convert to mm. We used the MRI acquisition parameters as a conversion factor, for example  $pixel\ size = 0.12mm$ .

We measured the cortical thickness along the entire contour. In order to get a reliable measure of the cortical thickness, we selected uniform cortical regions. Non-uniform values were probably outliers from the extremes, which due to the anatomy of the rat brain could have more fluctuations in the distance as shown in Fig.3b. To detect them we first calculated the median of all the values and we removed the points within the percentile 20, associated with outliers or extremes. Therefore, all these points were removed from the final result.

#### D. Statistical analysis

The above algorithm results in a set of cortical thickness measures including several slices of each subject. The cortical thickness of each subject was then obtained by doing a median of these values. Kruskal-Wallis tests were used to evaluate statistical differences between groups and times.

## IV. RESULTS AND DISCUSSION

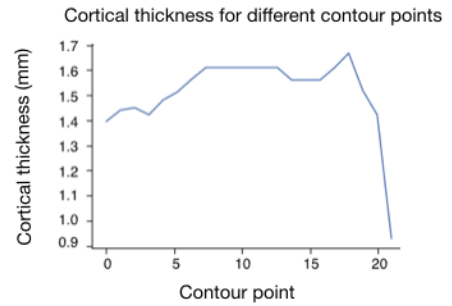


FIG. 5: Cortical thickness vs the points of the contour line for one subject (CTR) in the time point t1 and slice 18

The output values of the algorithm were the cortical thickness for each slice that survived the threshold for each time point and subject. One example of this results is shown in Fig.5, where we can see the distance for the different points along the cortex boundary. To get a suitable cortical thickness measure, it was necessary to:

1. Select the slices that survive the threshold. We can see in the Fig.6 that the slices 14–29 survived the threshold, however, only the slices 15–19 had stable values of cortical thickness, so we only kept these values for the analysis of the results.
2. We removed one subject (CTR) from the data as its cortical thickness is very different from the rest of subjects, as can be observed in the Fig.7.

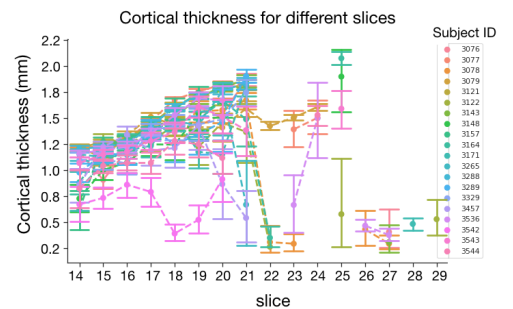


FIG. 6: Cortical thickness vs slices that survives the threshold for the different subjects of the study

In order to get only one value per subject and time we calculated the median for all the slices with the values that we obtained after removing the outliers, which we had found in the last checking of the algorithm results. We then analysed the behaviour of the CTR and the TgF344-AD group of rats based on these cortical thickness values.

We grouped the results into three measures to study them: right cortical thickness (Fig.8), left cortical thickness (Fig.9) and bilateral cortical thickness (Fig.10),

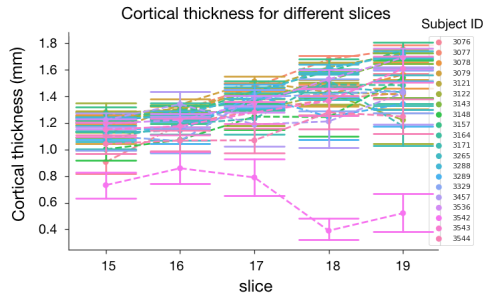


FIG. 7: Cortical thickness vs the selected slices for the different subjects of the study

which conducted the study putting all the measurements together. We could see that in the three cases they followed a similar behaviour as we expected.

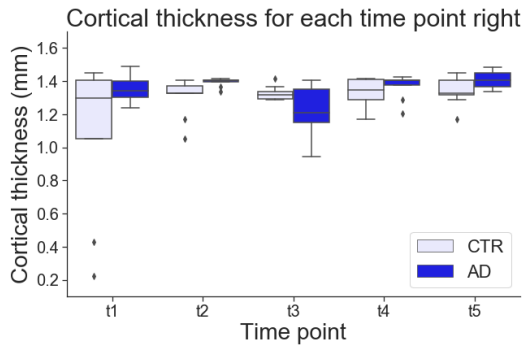


FIG. 8: Cortical thickness right vs time point

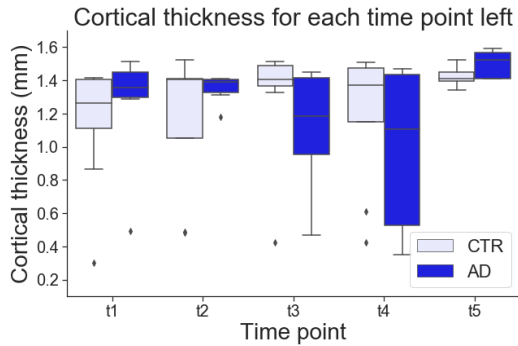


FIG. 9: Cortical thickness left vs time point

In the literature values of the cortical thickness between 1.2 – 1.7 mm for adolescent rats and 1.1 – 1.6 mm for adult rats were reported [8]. In our study, we obtained the values shown in Table I. These results are on the same order of magnitude, which confirms the correct estimation of our developed algorithm.

The results of Kruskal-Wallis test showed that the brain cortex changed over time and differed between groups at time point 2 for the right hemisphere (Fig.11, 12, 13).

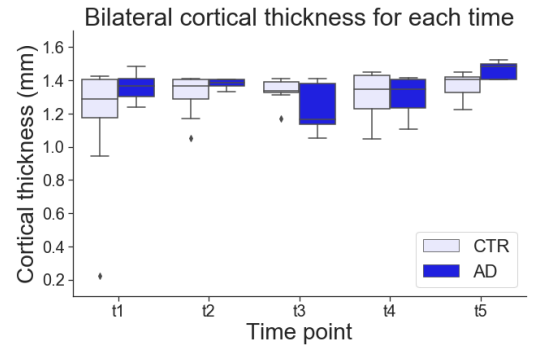


FIG. 10: Bilateral cortical thickness vs time point

	<i>Cth R</i> (mm)	<i>Cth L</i> (mm)	<i>Cth RL</i> (mm)
t1 CTR	1.30 [1.05-1.41]	1.26 [1.11-1.41]	1.29 [1.18-1.41]
t2 CTR	1.33 [1.33-1.37]	1.41 [1.05-1.41]	1.37 [1.29-1.41]
t3 CTR	1.32 [1.29-1.34]	1.41 [1.36-1.49]	1.34 [1.33-1.39]
t4 CTR	1.35 [1.29-1.41]	1.37 [1.15-1.48]	1.35 [1.23-1.41]
t5 CTR	1.33 [1.32-1.41]	1.41 [1.40-1.45]	1.41 [1.33-1.42]
t1 AD	1.34 [1.30-1.40]	1.36 [1.30-1.45]	1.37 [1.30-1.41]
t2 AD	1.41 [1.40-1.41]	1.39 [1.33-1.41]	1.39 [1.37-1.41]
t3 AD	1.21 [1.15-1.35]	1.18 [0.95-1.42]	1.17 [1.13-1.38]
t4 AD	1.41 [1.38-1.41]	1.11 [0.53-1.44]	1.35 [1.23-1.41]
t5 AD	1.41 [1.37-1.45]	1.52 [1.41-1.57]	1.48 [1.41-1.50]

TABLE I: The median for the cortical thickness in each time point for the three cases studied: cortical thickness right (*Cth R*), cortical thickness left (*Cth L*) and bilateral cortical thickness (*Cth RL*). Interquartile intervals are indicated with square brackets.

Right pvalues for time point CTR					
	t1	t2	t3	t4	t5
t1	0.4	0.627	0.53	0.43	0.4
t2	0.356	0.014*	0.69	0.561	0.766
t3	0.093	0.012*	0.148	0.809	0.628
t4	0.744	1.908	0.024*	0.559	0.958
t5	0.361	0.699	0.027*	0.547	0.088
AD	t1	t2	t3	t4	t5

FIG. 11: pvalues from the Kruskal-Wallis test for the right results. Significant differences  $p < 0.05$  indicated with \*. Upper diagonal for CTR and lower diagonal for AD temporal difference. Diagonal for CTR-AD group difference.

## V. FUTURE WORK

In the algorithm created we can make some future improvements to achieve a better measure of the cortical thickness. Some of our proposals are:

1. To get a more region-specific study. For the moment the algorithm gets the results of the entire cortex, in the future we would like to study specific

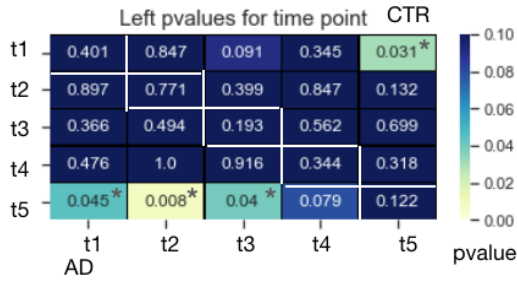


FIG. 12: p-values from the Kruskal-Wallis test for the left results. Significant differences  $p < 0.05$  indicated with \*. Upper diagonal for CTR and lower diagonal for AD temporal difference. Diagonal for CTR-AD group difference.

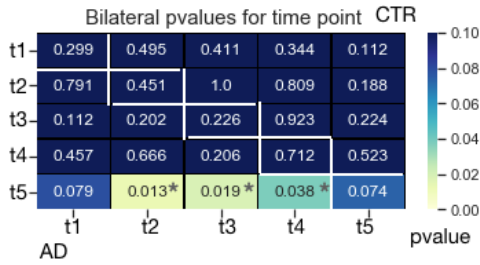


FIG. 13: p-values from the Kruskal-Wallis test for the bilateral results. Significant differences  $p < 0.05$  indicated with \*. Upper diagonal for CTR and lower diagonal for AD temporal difference. Diagonal for CTR-AD group difference.

regions of the cortex.

2. To find a better approximation of the initial threshold to select the slices of interest.
3. To get more contour points so we will have more measures for each slice. We expect that a higher number of points will improve the computation of the median value as a measure.

4. To remove the extremes of the cortex before getting measures since we know in advance that we do not want to measure the cortical thickness in this part when considering an averaging measurement of the cortical thickness. They can be used to explore local measurements.
5. To modify the algorithm to be able to measure the cortical thickness in a 3D approach, taking into account the resolution in each direction.

## VI. CONCLUSIONS

We have created an algorithm that measures automatically the cortical thickness from rat brain MRI data and we have used it to obtain individual measures. Our algorithm succeed in measuring the cortical thickness giving that we obtained values comparable to others reports [8].

The algorithm is a combination of scripts from the FSL loadbox and in-house created algorithms with Python. Moreover, it is totally automatic as it does not need human interaction to obtain correct measures once the parameters are setted. The algorithm is designed using general properties so it can be use with other rat brain MRI studies with other objectives. In our case we used it to study a transgenic model of AD, and observed the temporal evolution of the cortical thickness.

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